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Review

DNA vaccines against cytomegalovirus: current progress

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Abstract

The development of a vaccine for the prevention of primary cytomegalovirus (CMV) infection is a major public health priority. Live attenuated virus, recombinant viral vector, recombinant protein and peptide vaccines have been studied as potential vaccine candidates. In recent years, DNA vaccination strategies have been developed for many pathogens, including CMV. This review aims to bring together many aspects of this relatively new vaccine technology as applied to current research into the development of vaccines against CMV. © 2002 Elsevier Science B.V. and International Society of Chemotherapy. All rights reserved.

Keywords: DNA vaccines; Cytomegalovirus; Antibody; CTL; Protection

1. Introduction

The β-herpesvirus, cytomegalovirus (CMV) infects the majority of individuals during their lifetime yet results in disease only in those whose immune system is immature or impaired by immunosuppressive drugs or human immunodeficiency virus. This virus infects 0.3–2.4% of neonates born in different countries making it the most important cause of intrauterine infection [1]. There is an estimated 1% chance of developing primary CMV infection whilst pregnant in women who enter pregnancy seronegative for CMV [2,3].

The development of a CMV vaccine for the prevention of primary CMV infection is thus a major public health priority. A current report from the Institute of Medicine strongly supports the development of a CMV vaccine based on the economic impact of the disease caused by this virus [4]. A study published recently by our group using a mathematical modelling approach calculated that the critical vaccination proportion required for eradication of CMV in the developed world lies between 59 and 62% demonstrating that even if a putative vaccine were only 80–90% effective in preventing primary infection, CMV could be eradicated from the population by the immunisation of 66–75% of the population, a target easily achievable given a 90% current routine paediatric immunisation rate [5].

Of the 200 genes encoded by the CMV genome [6], only a small proportion are thought to comprise the targets of immune responses necessary for protection against CMV [7]. The neutralizing antibody response against HCMV is predominantly directed against a single protein, glycoprotein B (gpUL55) [8,9] while the tegument protein pp65 (ppUL83) is a major target of the cellular immune response [10–12]. These antigens have formed the basis of the majority of vaccine candidates thus far, which encompass their use within recombinant viral vectors [13,14], recombinant protein vaccines [15] and peptide vaccines [16]. Other candidate vaccine antigens have been proposed or studied for inclusion into a CMV vaccine. HCMV gH is a major target for complement-independent neutralizing antibody response in animals and humans and is thus a candidate antigen [17]. gH requires the assistance of another protein, gL for transport to the cell surface [18] which may thus need to be included in the vaccine preparation. It has recently been shown that 62% of sera from HCMV-seropositive donors reacted with the HCMV gM–gN complex, which thus may represent a major antigenic target and vaccine candidate [19,20]. With regards the generation of cellular responses, the non-structural IE1-exon4 protein has been shown to be an important CTL target [21].

Over the last few years, DNA vaccines have been shown to be effective inducers of cellular and humoral responses against viral antigens [22,23]. This review aims to bring together many aspects of this new vaccine

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technology as applied to current research into the development of vaccines against CMV.

2. Evolution of a CMV DNA vaccine

The first report in the literature detailing the use of DNA vaccination for CMV involved the immunisation of mice with plasmid DNA encoding the tegument protein pp65 of HCMV [24]. In this study, BALB/c mice were immunised i.m. with two plasmid constructs in which pp65 expression was controlled by either the human β-actin promoter or the HCMV immediate-early (IE) promoter. After 100 μg booster given 5 weeks post initial immunisation, approximately 60% of mice exhibited antibody responses to pp65. Antibody titres were found to be higher in mice immunised with pp65 under the control of the HCMV IE promoter, which was thought to be due to higher levels of expression. This study generated considerable interest in the field of CMV vaccinology and provided a basis for future investigations into the use of plasmid DNA constructs as mediators of immune responses against CMV. What this study did not present was any data on the ability of this pp65 DNA vaccine to induce specific cell-mediated immunity.

3. Demonstration of the protective capacity of CMV DNA vaccines

Using the murine cytomegalovirus (MCMV) IE gene 1 (pp89), the major target for CD8 T-cells in the murine CMV model, under the control of strong enhancer/promoter sequences from the HCMV IE gene, Gonzalez Armas et al. showed that immunisation with this construct could confer protection against MCMV infection [25]. This DNA vaccine elicited the production of pp89-specific CTLs which afforded a 45% (mean) protection against lethal challenge and highly significant reductions in spleen and salivary gland viral titres relative to control immunisations (up to 66-fold). A subsequent DNA vaccination study from the same group identified a new viral gene product, M84, a non-structural protein with amino acid homology to HCMV pp65 which confers protection against viral replication in BALB/c mice spleens [26,27].

4. CMV DNA vaccines that stimulate both arms of the immune system

The generation of both humoral and cell mediated immunity are believed to be necessary requisites for an effective CMV vaccine [7]. Endresz et al. used a 'cocktail' DNA vaccine approach comprising plasmids encoding two HCMV (Towne strain) proteins, gB and pp65 to generate gB-specific neutralizing antibody and pp65-specific CTL responses in BALB/c mice [28]. Two gB DNA vaccine constructs were made incorporating full-length gB and a truncated gB lacking the transmembrane domain. Mice immunised with the truncated gB exhibited higher titres of ELISA and neutralising antibodies than did those receiving full-length gB. Antibodies to full-length gB were predominantly of the IgG2a isotype, whereas those to truncated gB were mainly IgG1, suggested by the authors to be due to different antigen presentation mechanisms. All mice co-immunised with full-length gB and pp65 constructs developed gB- and pp65 specific ELISA antibodies that were shown to persist over the 31-week assay period. Of the co-immunised mice sacrificed for CTL analysis, 80% developed pp65-specific CTL responses as demonstrated in a Cr-release assay. The generation of gB-specific CTL activities was not tested in this study.

This study showed a lack of interference between the gB and pp65 constructs when co-immunised into mice at the same site and thus paved the way for future work involving a multi-target approach to DNA vaccination against CMV.

Guinea pig cytomegalovirus (GPCMV) is an excellent animal model for the development of vaccines against congenital CMV infection as it causes disease in utero. Schleiss et al. has used this model to test plasmid constructs targeting two GPCMV antigens, gB and pp65 [29]. This study showed that after epidermal immunisation of guinea pigs, all gB-immunised animals produced anti-gB antibody titres comparable to natural infection. Antibody responses to pp65 were also demonstrated in all immunised animals.

5. Enhancement of the immune response to a CMV DNA vaccine

Many groups have attempted to enhance the immune response to DNA vaccines by methods including the co-administration of various immunomodulators (cytokines, chemokines, costimulatory molecules), delivery of plasmids in liposomes and the use of experimental adjuvants [30]. The downside of these approaches with regards to prophylactic and therapeutic vaccine applications is that they are currently unlicensed for human use. It has recently been shown that negatively charged aluminium salts, currently licensed for use in humans, can be employed to greatly enhance antibody responses to DNA vaccine-encoded genes [31]. We have used a gel formulation of aluminium phosphate to boost IgG responses to an Ad169 HCMV gB DNA vaccine. Mice given a single boost of gB plasmid at week 5 post initial immunisation attained a geometric mean IgG titre (de-

termined by immunofluorescence) of 1/5120 at week 6 (compared with 1/640 in mice given plasmid without adjuvant). Mice given two boosts (weeks 5, 10) had a geometric mean IgG titre of 1/17 800 at week 11 (1/8900 without adjuvant). Immunofluorescence has been used previously in a gB DNA vaccine study in BALB/c mice to determine IgM and IgG titres [32]. Mice were immunised with 100 μg plasmid with two further boosters given at weeks 2 and 4, respectively. Geometric mean IgM antibody titre at its peak level (1–2 weeks after second booster) was shown to be 1/54, and IgG titre at its peak level (3 weeks after second booster) was shown to be 1/262. This vaccine also induced a neutralising antibody response with a percentage reduction of input infectivity (in 1:100 diluted sera) of 74.5% in mice after the two boosts.

As regards humoral responses, the potency of DNA vaccines is often inferior to that of a protein-based vaccine based around homologous antigens. Our ongoing studies in CMV indicate that the HCMV-gB/aluminium phosphate formulation could represent an effective humoral immunity-inducing component of a multi-target DNA vaccine against this virus.

The consecutive use of a DNA vaccine priming agent and an attenuated viral vector or recombinant protein boost, involving similar heterologous antigens has proved effective for the generation of high levels of humoral and cell mediated immunity [33–35]. Attenuated recombinant viral vectors have been employed previously as vaccine candidates against HCMV either alone or as priming agents. Berencsi et al. demonstrated the induction of a murine CTL response specific for HCMV gB by adenovirus and vaccinia virus recombinants expressing gB [13]. Adler et al. have used a canarypox vector expressing gB to prime for antibody responses to a live attenuated CMV vaccine [36]. A recent study has investigated the possibility of using a gB DNA vaccine construct to prime humoral responses to a gB subunit preparation in BALB/c mice [37]. Priming with a construct encoding the secreted form of gB followed by a gB subunit boost resulted in the generation of high-titre antibody responses similar in type to those obtained previously by this group with a canarypox-gB prime/gB subunit boost. Levels of IgG2a antibodies, induced at low levels by the secreted gB DNA vaccine alone, were significantly increased by boosting with the gB subunit suggesting the activation of Th1 responses.

In conclusion, research into the development and evaluation of candidate DNA vaccines against CMV is gradually gaining momentum and with the publication of the Institute of Medicine report it is hoped that some of these vaccines will shortly be investigated in human Phase I clinical trials.

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